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Interactions between QTL SAP6 and SU91 on resistance to common bacterial blight in red kidney bean and pinto bean populations

G. J. Vandemark · D. Fourie · R. C. Larsen · P. N. Miklas

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Abstract Breeding efforts to improve resistance in dry bean to common bacterial blight (CBB) have focused on applying marker assisted selection strategies. We examined the interaction between two independent QTL (quantitative trait loci), SAP6 and SU91, on the expression of resistance to CBB in a pinto bean F_2 population and dark red kidney bean F_2 population. The disease reaction for each F_2 population was scored at several time points after inoculation. The dominant SCAR markers SAP6 and SU91 were simultaneously genotyped as codominant markers using a multiplex real-time PCR assay. For both populations, at each time point plants having genotypes of (su91 su91//SAP6 SAP6); (su91 su91//SAP6 sap6), and (su91 su91//sap6 sap6) were significantly more susceptible to CBB than plants with any of the six other possible genotypes (SU91 _//_ _). Only two examples were observed across both populations and all time points in which mean disease reactions were significantly different between any of the six (SU91 _//_ _) genotypes. No significant differences were observed between SU91 SU91 and SU91 su91 genotypes at any time point for either F_2 population. Similarly, no significant differences in CBB reaction were observed among the three SAP6 genotypes for plants that had the su 91/su91 genotype. The results indicate CBB resistance in these two populations is primarily conditioned by the presence of at least a single copy of the SU91 QTL. The effects of pathogen isolate and population on discerning the influence of QTL on CBB resistance are discussed.

Keywords Disease resistance · *Phaseolus vulgaris* · *Xanthomonas axonopodis*

G. J. Vandemark (✉)
US Department of Agriculture, Grain Legume Genetics and Physiology Unit, Pullman, WA 99164, USA
e-mail: george.vandemark@ars.usda.gov

D. Fourie
ARC Grain Crops Institute, Potchefstroom, South Africa

R. C. Larsen · P. N. Miklas
US Department of Agriculture, Vegetable and Forage Crop Research Unit, Prosser, WA 99350, USA

Introduction

Common bacterial blight (CBB), caused by *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*), is a globally distributed disease of common beans (*Phaseolus vulgaris* L.) that results in severe economic losses, especially in tropic and subtropic regions (Coyne and Schuster 1974). Susceptible cultivars can suffer losses from CBB that exceed 40% of yield potential (Saettler 1991). The pathogen *Xap* is seed-transmitted and contaminated seed is typically the primary source of inoculum for both local and global distribution of CBB disease (Saettler 1991). The pathogen can

overwinter in infested plant debris and can also grow as an epiphyte on leaves of non-host crops and weeds (Saettler 1991). These alternate sources of inoculum can also serve to initiate the disease cycle in fields that are planted with pathogen-free seed, so control measures based on crop rotation have limited efficacy. Control measures based on chemical applications have not been shown to significantly increase seed yield or reduce pod infection (Weller and Saettler 1976). The most effective method for controlling CBB is to grow resistant bean cultivars (Singh and Munoz 1999).

Resistance to CBB in common bean is primarily inherited quantitatively (Beebe 1989; Silva et al. 1989; Miklas et al. 1996; Yu et al. 1998). Considerable efforts have been made to use molecular markers to select for genotypes that are resistant to CBB. The use of molecular markers potentially has several advantages over selection based on visual assessment of disease reaction, including the ability to screen for resistant genotypes at the seedling stage and avoidance of confounding effects of environmental factors on disease expression. At least 22 different quantitative trait loci (QTL) conditioning resistance to CBB have been identified across all 11 linkage groups of common bean (Miklas et al. 2006a). These QTL are derived from a wide range of common bean breeding lines and also from tepary bean (*P. acutifolius* L.) and scarlet runner bean (*P. coccineus* L.) (Miklas et al. 2006a).

The most comprehensive characterizations of the interaction between different QTLs implicated in resistance to CBB have considered the interactions between the QTL-linked markers BC420 (Yu et al. 1998) and SU 91 (Pedraza et al. 1997). The BC420 and SU91 are both sequence characterized amplified region (SCAR) (Paran and Michelmore 1993) markers and are located on linkage groups B6 and B8, respectively. Both QTL derive from the common bean breeding line XAN 159 (Thomas and Waines 1984). This line was developed from a interspecific cross between common bean and tepary bean (PI 319443) which is the source of CBB resistance in XAN 159 (Kolkman and Michaels 1994). Park et al. (1999) examined the influence of the randomly amplified polymorphic DNA (RAPD) (Williams et al. 1990) markers BC420.900 and E41150, from which BC420 and SU91 were, respectively, derived, on the expression of CBB resistance. An additive effect in the direction of increased disease resistance was observed for bean genotypes that possessed both markers. O'Boyle et al. (2007) reported that bean genotypes that possessed both BC420 and SU91 had less resistance to CBB than genotypes that possessed only one of either marker and suggested the possibility of an epistatic interaction between the two QTLs.

Recently Vandemark et al. (2008) examined the interaction between BC420 and SU91 in the expression of resistance to CBB in common bean using a near isogenic population derived from a cross between XAN 159 (resistant) and Teebus (susceptible). Plants were genotyped using an approach based on real-time PCR (Holland et al. 1991; Heid et al. 1996) that can be used to co-dominantly interpret plant genotypes for dominant SCAR markers (Vandemark and Miklas 2002, 2005). Using real-time PCR, it was possible to assign BC_6:F_2 plants to all nine possible genotypes. Reaction to CBB in BC_6:F_2 plants was characterized by an epistatic interaction between BC420 and SU91. Plants that were homozygous recessive at both loci (bc420 bc420/su91 su91) and plants had at least a single copy of BC420 but were homozygous recessive for SU91 were susceptible to CBB; plants that had at least one copy of SU91 but were homozygous recessive for BC420 expressed an intermediate disease reaction, and the highest level of disease resistance was conferred by genotypes with at least a single resistance copy of both SU91 and BC420 (Vandemark et al. 2008). Segregation for resistance among BC_6:F_3 plants derived from BC_6:F_2 plants that were heterozygous for both QTL did not deviate significantly from expected ratios of 9 resistant: 3 moderately resistant: 4 susceptible, which was consistent with a recessive epistatic model of inheritance between two loci. These results suggested that breeders would maximize gains in resistance to CBB by selecting plants that were fixed for both QTL.

A prerequisite for effectively combining QTLs from different sources is to determine how different QTLs interact in specific genetic backgrounds to condition disease resistance. Another QTL of importance in conditioning resistance in bean to CBB is the SCAR marker SAP6 (Miklas et al. 2000), which is located on linkage group B10 and is derived from the great northern landrace cultivar Montana No. 5. The presence of SAP6 accounted for 35% of the variation in resistance to CBB in an F_2 population derived from the cross Montana No.5/Othello (Miklas et al. 2003).

Like SU91 and BC420, SAP6 is a dominant SCAR marker, and genotypes that are homozygous for SAP6 have historically been distinguished from heterozygous genotypes by the process of observing segregation for resistance to CBB in progeny of plants that have at least a single copy of the QTL. It has not been possible to use dominant SCAR markers to determine the allelic dosage effects of different numbers (one or two) of individual QTL on levels of resistance to CBB. Additionally, it is not possible to use dominant SCAR markers to investigate the effects of different numbers of copies of multiple QTL on disease resistance. The objectives of this study were to develop a multiplex real-time PCR assay that could be used to simultaneously genotype bean seedlings for SAP6 and SU91 and to use the assay to examine the genetic interaction between SAP6 and SU91 QTL on expression of resistance to CBB in two different F_2 populations.

Materials and methods

Plant materials

Two populations were examined in this study. One was a pinto bean population consisting of 135 F_2 plants from a cross between 'Othello' (sap6 sap6// su91 su91; susceptible to CBB) (Burke et al. 1995) and ABCP-8 (SAP6 SAP6//SU91 SU91; resistant to CBB) (Mutlu et al. 2005). ABCP-8 was developed by backcross breeding and marker assisted selection (Mutlu et al. 2005) for both SU91, derived from the donor parent XAN 159, and SAP6, derived from the recurrent parent Chase pinto. ABCP-8 was more resistant to CBB (6% infection in field and greenhouse tests) than the recurrent parent Chase (33% in field and 46% in greenhouse) and the susceptible check Othello (59% in field and 100% in greenhouse) (Mutlu et al. 2005).

The second population consisting of 146 F_2 plants was obtained from a cross between dark red kidney beans, 'Red Hawk' (sap6 sap6//su91 su91; susceptible to CBB) (Kelly et al. 1998) and USDK-CBB-15 (SAP6 SAP6//SU91 SU91; resistant to CBB). USDK-CBB-15 was developed from a modified backcrossing scheme (dark red kidney*4/XAN 159) which employed marker-assisted selection for CBB resistance QTL SU91 derived from XAN 159 and the QTL SAP6 derived from 'Montcalm' dark red kidney (Miklas et al. 2006b).

Real-time PCR primers and probes

The nucleotide sequence of SAP 6 (806 bp, GENBANK # FJ471534) was analyzed with Primer Express software (Applied Biosystems, Foster City, CA) to identify sequences for real-time PCR primers and probes. The primers and the fluorochrome-labeled probe used in this study are as follows: forward primer SAP6F239, 5′-d-AAATAAACATTAATCTCCTAACCTCAGACA-3′; reverse primer SAP6R340, 5′-d-TCGATCATATGGTGAAATGGTCTT-3′; and probe SAP6P273, 5′-d-TCAATGAAACTGATAAGTTC-3′. The 5′ terminus of the probe (TaqMan; Applied Biosystems) was labeled with the fluorochrome 6-carboxyfluorescein (6-FAM). A non-fluorescent quencher coupled with a minor groove binder (MGB) was attached to the 3′ terminus. The primer/probe set SAP6F239-SAP6P273-SAP6R340 amplified a 102 bp fragment.

The real-time PCR primers and probe for amplifying SU91 (669 bp; GENBANK #EF553636) were as previously described (Vandemark et al. 2008): forward primer SU91F2, 5′-d-CACATCGGTTAACATGAGTGATTTC-3′; reverse primer SU91R86, 5′-d-CACACAAAGGAGGGATAAAAGAGATAA-3′; and probe SU91P34, 5′-d-CATATATCATCGCCTATTGTGT-3′. The fluorochrome VIC (Applied Biosystems) was attached to the 5′ terminus of the probe (TaqMan; Applied Biosystems) and a non-fluorescent minor groove binding quencher (MGB) was attached to the 3′ terminus. The primer/probe set SU91F2- SU91P34- SU91R86 amplified an 85 bp fragment.

Real-time PCR assays

In separate multiplex reactions for each plant sample, the primer/probe set SAP6F239-SAP6P273-SAP6R340 was used to amplify the 102 bp amplicon from SAP6 and SU91F2- SU9134- SU91R86 was used to amplify the 85 bp fragment from SU91. PCR was done in 50 µl reactions containing 100 ng genomic DNA, 450 nM each primer (SAP6F239, SAP6R340, SU91F2, and SU91R86), 125 nM each fluorochrome-labeled probe (SAP6P273 and SU91P34) and 25 µl of 2× TaqMan™ Universal PCR Master Mix (Applied

Biosystems). PCR and detection of fluorescence were performed using the GeneAmp 7300 Sequence Detection System (Applied Biosystems). All PCR was conducted using a thermocycling profile consisting of an initial cycle of 2 min at 50°C, followed by a single cycle of 10 min at 95°C, and then 40 cycles of 15 s at 95°C and 1 min at 60°C. For each DNA sample, two replicate real-time PCR reactions were performed with each primer/probe set. ddH_2O was substituted for 100 ng DNA in PCR to verify that reagents were not contaminated by template DNA. Standard curves were generated for SAP6 and SU91 using three replicate reactions each of 5, 25, 50, 100, and 200 ng of purified genomic DNA of the homozygous parents ABCP-1 and USDK-CBB-15 (SAP6 SAP6//SU91 SU91). The null genotype parents Othello and Red Hawk (sap6 sap6//su91 su91) were amplified with each primer/probe set in replicate reactions to confirm that the primer/probe sets did not amplify DNA from plants that did not possess at least one copy of each QTL.

Determining plant genotype with real-time PCR

For each population, 10 remnant F_1 plants (SAP6 sap6//SU91 su91) were examined to determine heterozygote probability distributions for SAP6 and SU91. Three replicate PCR reactions were performed on each F_1 plant for both QTL. The mean (y) and standard deviation (σ_y) for the F_1 plants were determined and the data was examined for a normal distribution with the Shapiro–Wilk test (Shapiro and Wilk 1965). Once a normal distribution was verified, a 99% probability distribution for heterozygotes was estimated for SAP6 and SU91 using the formula $y \pm 2.58\sigma_y$ (Walpole and Myers 1978).

The genotype of each F_2 plant for SAP6 and SU91 was determined based on the mean of two real-time PCR reactions for each marker. Plants with means within the 99% heterozygote probability distribution for a QTL were considered to be heterozygous for the QTL. Plants whose means fell to the right of the heterozygote probability distribution were considered homozygous for the QTL. Plants for which no amplification was detected were considered to be homozygous recessive for the QTL. Chi-square (χ^2) analysis was performed to determine if observed results were significantly different than expected segregation ratios of 1 (SAP6 SAP6): 2 (SAP6 sap6): 1 (sap6 sap6) and 1 (SU91 SU91): 2 (SU91 su91): 1 (su91 su91). The independent assortment of alleles at both QTL was also examined with χ^2 analysis to determine if observed results were significantly different than the expected segregation ratios of 1 (SAP6 SAP6//SU91 SU91): 2 (SAP6 SAP6//SU91 su91): 1 (SAP6 SAP6//su91 su91): 2 (SAP6 sap6//SU91 SU91): 4 (SAP6 sap6//SU91 su91): 2 (SAP6 sap6//su91 su91): 1 (sap6 sap6//SU91 SU91): 2 (sap6 sap6//SU91 su91): 1 (sap6 sap6//su91 su91).

Screening plants for resistance to CBB

Disease reaction of plants in response to inoculation by *Xap* was assessed for 10 plants of each parent, 10 F_1 plants for each population, 135 F_2 plants from the cross Othello × ABCP-8, and 146 F_2 plants from the cross Red Hawk × USDK-CBB-15. Seeds were planted in 20 L plastic bags (one seed per bag) in sterile soil and maintained in a greenhouse at 18°C night/28°C day. Inoculum (10^8 colony forming units (CFU)/ml) was prepared by suspending 48-to 72-h-old cultures of South African *Xap* isolates Xf260 and Xf410 in sterile distilled water, determining colony density with a spectrophotometer, and mixing an equal number of both *Xap* isolates. Xf260 and Xf410 were isolated from field grown beans and have been used previously to examine how the QTLs BC420 and SU91 interact to condition resistance to CBB (Vandemark et al. 2008). The multiple needle inoculation method (Andrus 1948) was used to inoculate the first fully expanded trifoliate leaves of 14–20 days old plants. Inoculated plants were maintained in a greenhouse at 18°C night/28°C day and rated for CBB infection at 7, 10, 14, and 18 days after inoculation (DAI) using a 1–9 scale (Aggour et al. 1989) to describe disease symptoms: 1 = no necrotic lesions and/or chlorosis; 2–3 = 1–25.5 leaf area affected; 4–6 = 26–64.5% leaf area affected, and 7–9 = 65–100% leaf area affected.

Results

Determining genotypes for SAP6 and SU91 in an F_2 pinto bean population (Othello × ABCP-8)

Standard curves for both primer/probe had R^2 values >0.99 for the relationship between the $\log_{10}$ of the

initial DNA quantity and C_T value for all sets of reactions. Fluorescence above background was not detected for SAP6 or SU91 in the homozygous recessive parent Othello (sap6 sap6//su91 su91) (not shown).

Real-time PCR results for the 10 F_1 plants based on the amplification of 100 ng DNA with primer/probe set SAP6F239-SAP6P273-SAP6R340 had a group mean and standard deviation of 53.53 ± 11.59. Results are expressed without units since the 102 bp fragment amplified represents such as small fraction of the entire bean genome. Analysis of F_1 data with the Shapiro–Wilk test indicated that the data was normally distributed ($N = 30$, $W = 0.94$, Prob < $W = 0.11$). Based on these results the 99% heterozygote confidence interval was determined to be 24.09–82.97.

Among 135 F_2 plants, the mean of two real-time amplification reactions for SAP6 fell to the right of the 99% heterozygote confidence interval for 47 plants, which were considered to be homozygous dominant (SAP6 SAP6). A total of 56 plants had mean PCR values that fell within the heterozygote confidence interval and were considered heterozygous (SAP6 sap6). The mean real-time PCR values for homozygous and heterozygous F_2 plants based on the amplification of 100 ng genomic DNA was 118.03 and 55.97, respectively. No amplification was detected for 32 plants, which were considered homozygous recessive (sap6 sap6). The observed genotypic ratios deviated significantly from the expected 1:2:1 ratios ($\chi^2 = 7.25$; $df = 2$; $P = 0.027$).

For SU91, real-time PCR results for 10 F_1 plants based on the amplification of 100 ng DNA with primer/probe set SU91F2- SU9134- SU91R86 had a group mean and standard deviation of 54.92 ± 10.02. The Shapiro–Wilk test indicated that the F_1 data was normally distributed ($N = 30$, $W = 0.94$, Prob < $W = 0.10$). Based on these results the 99% heterozygote confidence interval was determined to be 29.47–80.37. The mean real-time PCR values for homozygous and heterozygous F_2 plants based on the amplification of 100 ng genomic DNA was 110.19 and 57.23, respectively. In total, 41 homozygous dominant (SU91 SU91), 57 heterozygous (SU91 su91) and 37 homozygous recessive (su91 su91) genotypes were resolved with real-time PCR. The results did not deviate significantly from the expected 1:2:1 segregation ratios ($\chi^2 = 3.51$; $df = 2$; $P = 0.174$).

For both primer/probe sets, a value of 2.0 would be expected for the ratio of PCR results for homozygous plants/PCR results for heterozygous plants in the idealized conditions characterized by completely efficient PCR reactions performed using DNA samples that were quantified with absolute precision. For primer probe sets SAP6F239-SAP6P273-SAP6R340 and SU91F2- SU9134- SU91R86 the ratio of means for homozygous plants/heterozygous plants was 118.03/55.97 = 2.11 and 110.19/57.23 = 1.93, respectively, for the Othello × ABCP-8 F_2 population. Results with both primer/probe sets fell within 6% of the idealized ratio of 2.0, suggesting that a high degree of precision was maintained throughout the processes of DNA quantification and real-time PCR reactions.

The observed numbers of F_2 plants (Othello × ABCP-8) in each of nine possible genotype classes based on the results of real-time PCR assays for SAP6 and SU91 are presented in Table 1. Chi-square analysis indicated that the observed ratios were significantly different from expected genotypic ratios of 1 (SAP6 SAP6//SU91 SU91): 2 (SAP6 SAP6//

Table 1 Means separation of CBB response among F_2 plants (Othello/ABCP-8; $N = 135$) genotyped for SAP6 and SU91

Genotype[a]	*N*	Mean CBB reaction[b]		
		7 DAI[c]	10 DAI	14 DAI
sap6 sap6//su91 su91	8	2.50 A	7.00 A	8.38 A
SAP6 sap6//su91 su91	19	2.16 A	6.00 B	8.26 A
SAP6 SAP6//su91 su91	9	2.33 A	6.56 AB	8.44 A
sap6 sap6//SU91 su91	17	1.24 B	2.97 CD	4.94 B
SAP6 sap6//SU91 su91	20	1.30 B	3.70 C	4.93 B
SAP6 SAP6//SU91 su91	21	1.38 B	3.28 CD	4.14 B
sap6 sap6//SU91 SU91	7	1.29 B	3.00 CD	4.86 B
SAP6 sap6//SU91 SU91	17	1.24 B	2.91 D	4.26 B
SAP6 SAP6//SU91 SU91	17	1.12 B	3.0 C D	4.00 B
Othello	12	2.42	5.58	8.56
F_1 (Othello/ABCP-8)	5	1.20	2.60	3.70
ABCP-8	12	1.17	1.83	2.21

[a] Plants were genotyped for SAP6 using the primer/probe set SAP6F239-SAP6P273-SAP6R340 and for SU91 using the primer probe set SU91F2- SU9134- SU91R86

[b] Reaction to CBB was evaluated using the following scale: 1 = no necrotic lesions and/or chlorosis; 2–3 = 1–25.5 leaf area affected; 4–6 = 26–64.5% leaf area affected, and 7–9 = 65–100% leaf area affected

[c] DAI = Days after inoculation. Numbers in a column with the same letter are not significantly different ($P < 0.05$)

SU91 su91): 1 (SAP6 SAP6//su91 su91): 2 (SAP6 sap6//SU91 SU91): 4 (SAP6 sap6//SU91 su91): 2 (SAP6 sap6//su91 su91): 1 (sap6 sap6//SU91 SU91): 2 (sap6 sap6//SU91 su91): 1 (sap6 sap6//su91 su91) ($\chi^2 = 15.87$; $df = 8$; $P = 0.044$), suggesting that the two QTL are not independently assorting in this population (Table 1).

Determining genotypes for SAP6 and SU91 in an F_2 red kidney bean population (Red Hawk × USDK-CBB-15)

Standard curves for both primer/probe had R^2 values >0.99 for the relationship between the $\log_{10}$ of the initial DNA quantity and C_T value for all sets of reactions. Fluorescence above background was not detected for SAP6 or SU91 in the homozygous recessive parent Red Hawk (sap6 sap6//su91 su91) (Fig. 1).

Real-time PCR results for the 10 F_1 plants based on the amplification of 100 ng DNA with primer/probe set SAP6F239-SAP6P273-SAP6R340 had a mean and standard deviation of 47.68 ± 6.27. Analysis of F_1 data with the Shapiro–Wilk test indicated that the data was normally distributed ($N = 30$, $W = 0.95$, Prob < $W = 0.23$). Based on these results the 99% heterozygote confidence interval was determined to be 31.75–63.61. The mean real-time PCR values for homozygous and heterozygous F_2 plants based on the amplification of 100 ng genomic DNA was 86.95 and 44.57, respectively. The ratio of means for homozygous plants/heterozygous plants was 86.95/44.57 = 1.95. The following genotypes were determined based on real-time PCR: 31 homozygous dominant (SAP6 SAP6), 77 heterozygous (SAP6 sap6) and 38 homozygous recessive (sap6 sap6) genotypes were resolved with real-time PCR from this F_2 population. The results did not deviate significantly from the expected 1:2:1 genotypic ratios ($\chi^2 = 1.11$; $df = 2$; $P = 0.57$).

Real-time PCR results for the F_1 plants based on the amplification of 100 ng DNA with primer/probe set SU91F2- SU9134- SU91R86 had a mean and standard deviation of 50.03 ± 7.26. Analysis of F_1 data with the Shapiro–Wilk test indicated that the data was normally distributed ($N = 30$, $W = 0.93$, Prob < $W = 0.27$). Based on these results the 99% heterozygote confidence interval was determined to be 31.59–68.47. The mean real-time PCR values for homozygous and heterozygous F_2s based on the amplification of 100 ng genomic DNA was 113.5 and 56.99, respectively. The ratio of means for homozygous plants/heterozygous plants was 113.5/56.99 = 1.99, which was within 0.5% of an idealized ratio of 2.0. The following genotypes were determined based on real-time PCR for SU91: 42 (SU91

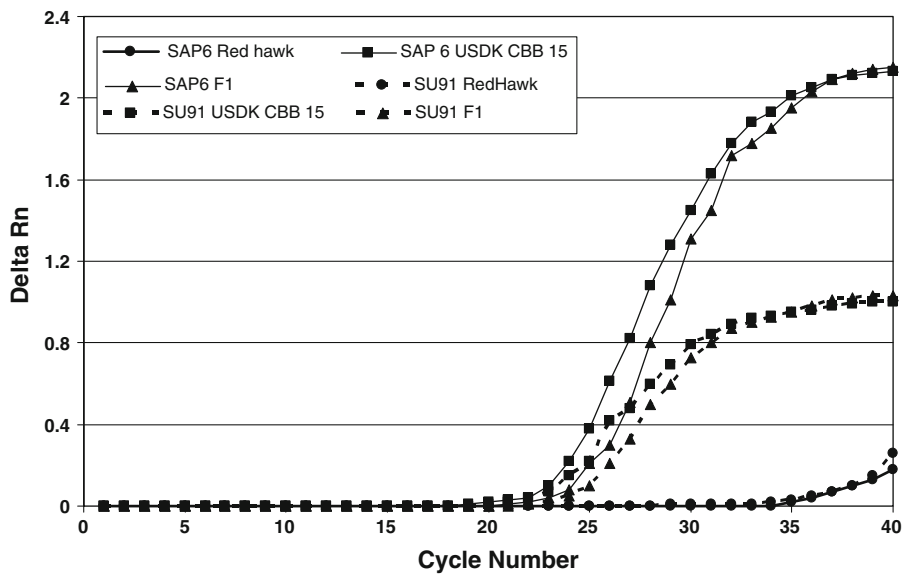

Fig. 1 Real-time amplification plot of total DNA isolated from leaves of individual plants of Red Hawk (sap6 sap6//su91 su91), USDK-CBB 15 (SAP6 SAP6//SU91 SU91) and their F_1 (SAP6 sap6//SU91//su91)

Table 2 Means separation of CBB response among F_2 plants (Red Hawk/USDK-CBB-15; $N = 146$) genotyped for SAP6 and SU91

Genotype[a]	*N*	Mean CBB reaction[b]			
		7 DAI[c]	10 DAI	14 DAI	18 DAI
sap6 sap6//su91 su91	11	2.36 B	5.18 B	6.91 B	7.80 A
SAP6 sap6//su91 su91	8	2.25 BC	5.13 B	6.50 B	7.29 A
SAP6 SAP6//su91 su91	5	3.2 A	6.20 A	8.40 A	8.00 A
sap6 sap6//SU91 su91	18	1.56 D	2.75 C	3.69 C	3.78 BC
SAP6 sap6//SU91 su91	42	1.61 D	2.64 C	3.26 C	3.37 C
SAP6 SAP6//SU91 su91	20	1.58 D	2.98 C	3.63 C	3.83 BC
sap6 sap6//SU91 SU91	9	1.56 D	2.89 C	3.11 C	3.25 BC
SAP6 sap6//SU91 SU91	27	1.78 CD	2.94 C	3.76 C	4.09 B
SAP6 SAP6//SU91 SU91	6	1.67 CD	2.75 C	3.75 C	4.08 BC
Red Hawk	12	3.18	6.18	8.18	8.25
F_1 (Red Hawk/USDK-CBB-15)	10	1.8	3.2	3.55	3.95
USDK-CBB-15	12	1.75	2.67	3.24	3.41

[a] Plants were genotyped for SAP6 using the primer/probe set SAP6F239-SAP6P273-SAP6R340 and for SU91 using the primer probe set SU91F2- SU9134- SU91R86

[b] Reaction to CBB was evaluated using the following scale: 1 = no necrotic lesions and/or chlorosis; 2–3 = 1–25.5 leaf area affected; 4–6 = 26–64.5% leaf area affected, and 7–9 = 65–100% leaf area affected

[c] DAI = Days after inoculation. Numbers in a column with the same letter are not significantly different ($P < 0.05$)

SU91): 80 (SU91 su91): 24 (su91 su91). The results did not deviate significantly from the expected 1:2:1 genotypic ratios ($\chi^2 = 5.78$; $df = 2$; $P = 0.06$).

The observed numbers of F_2 plants (Red Hawk × USDK-CBB) in each of nine possible genotype classes based on the results of real-time PCR assays for SAP6 and SU91 are presented in Table 2. Chi-square analysis demonstrated that the results did not significantly differ from expected genotypic ratios of 1 (SAP6 SAP6//SU91 SU91): 2 (SAP6 SAP6//SU91 su91): 1 (SAP6 SAP6//su91 su91): 2 (SAP6 sap6//SU91 SU91): 4 (SAP6 sap6//SU91 su91): 2 (SAP6 sap6//su91 su91): 1 (sap6 sap6//SU91 SU91): 2 (sap6 sap6//SU91 su91): 1 (sap6 sap6//su91 su91) ($\chi^2 = 14.27$; $df = 8$; $P = 0.07$), which indicates the two QTL are independently assorting in this population (Table 1).

Relationship between QTL genotypes and resistance to CBB

Means separations for reaction to CBB between the nine possible genotypes for 135 F_2 plants derived from the cross Othello × ABCP-8 are presented in Table 1 for several time points. The mean disease reactions of the resistant parent ABCP-8, F_1 plants, and the susceptible parent Othello are also presented. Othello (susceptible) and ABCP-8 (resistant) expressed expected disease reactions and at every time point disease was more severe in Othello. By 14 DAI, Othello had a mean disease reaction ≥7, which is considered susceptible to CBB. At all time points the mean CBB reaction of ABCP-8 was ≤3, which is considered a resistant reaction. Data for CBB reaction 18 DAI is not presented because by this time the majority of Othello and F_2 plants had lost their inoculated leaves.

At every time point the three genotypes that were homozygous recessive for SU91 (sap6 sap6//su91 su91); (SAP6 sap6//su91 su91), and (SAP6 SAP6//su91 su91) had significantly higher mean reactions (more susceptible) to CBB than the six other genotypes, all of which were either homozygous (_ _// SU91 SU91) or heterozygous (_ _//SU91 su91) for SU91. At 7 and 14 DAI, no significant differences in CBB reaction were observed between the six genotypes that had at least a single copy of SU91 (_ _// SU91 _). At 10 DAI, the only significant difference in CBB reaction among these six genotypes was that SAP6 sap6//SU91 SU91 was significantly lower (more resistant) than SAP6 sap6//SU91 su91. By 14 DAI, the mean CBB reactions of the six most resistant

Table 3 Means separation for reaction to CBB among F_2 plants genotyped for SU91 (A) and SAP6 (B)

	Mean CBB reaction[a]						
	F_2 (Othello × ABCP-8)			F_2 (Red Hawk × USDK-CBB-15)			
	7 DAI[b]	10 DAI	14 DAI	7 DAI	10 DAI	14 DAI	18 DAI
(A) SU91 genotype[c]							
su91 su91	2.24 A	6.32 A	8.30 A	2.50 A	5.38 A	7.08 A	7.63 A
Su91 su91	1.32 B	3.31 B	4.61 B	1.71 B	2.90 B	3.62 B	3.91 B
SU91 SU91	1.20 B	2.96 B	4.26 B	1.59 B	2.75 B	3.45 B	3.58 B
(B) SAP6 genotype							
sap6 sap6	1.56 A	3.98 A	5.78 AB	1.79 A	3.49 A	4.49 A	4.77 A
SAP6 sap6	1.57 A	4.24 A	5.86 A	1.73 A	3.01 A	3.77 A	3.97 B
SAP6 SAP6	1.47 A	3.81 A	4.91 B	1.85 A	3.45 A	4.42 A	4.22 AB

[a] Reaction to CBB was evaluated using the following scale: 1 = no necrotic lesions and/or chlorosis; 2–3 = 1–25.5 leaf area affected; 4–6 = 26–64.5% leaf area affected, and 7–9 = 65–100% leaf area affected

[b] DAI = Days after inoculation. Numbers in a column with the same letter are not significantly different ($P < 0.05$)

[c] Plants were genotyped for SAP6 using the primer/probe set SAP6F239-SAP6P273-SAP6R340 and for SU91 using the primer probe set SU91F2- SU9134- SU91R86

genotypes were all >3 but <7, which is considered an intermediate reaction, while the mean CBB reactions of the three most susceptible genotypes was ≥7, which is considered a susceptible reaction.

Means separations for reaction to CBB between possible genotypes for 146 F_2 plants derived from the dark red kidney cross (Red Hawk/USDK-CBB-15) are presented in Table 2 along with reactions of the resistant parent (USDK-CBB-15), F_1 plants, and the susceptible parent (Red Hawk). Red Hawk had a mean disease reaction ≥7, which is considered susceptible to CBB, by 14 DAI. USDK-CBB-15 had a mean CBB reaction >3 but <7 by 14 DAI, which is considered an intermediate disease reaction. Data is presented for 18 DAI because defoliation of inoculated leaves was rarely observed for either the F_2 plants or the susceptible parent Red Hawk. At every time point significantly lower CBB reactions were observed for the six genotypes that had at least a single copy of SU91 (_ _//SU91 _) than was observed for the three other genotypes that were all homozygous recessive for SU91 (_ _//su91 su91). At 7, 10 and 14 DAI no significant differences in CBB reaction were observed between the six genotypes that had at least a single copy of SU91. At 18 DAI, the only significant difference in CBB reaction among these six genotypes was that SAP6 sap6// SU91 SU91 was significantly higher (more susceptible) than SAP6 sap6//SU91 su91.

Means separations for reaction to CBB between plants having different genotypes of SU91 are presented in Table 3 for different time points for each F_2 population. For both F_2 populations the mean CBB reaction of plants that were homozygous recessive for SU91 (su91 su91) was significantly higher (more susceptible) than the other two genotypes at each time point. No significant differences were observed between SU91 SU91 and SU91 su91 genotypes at any time point for either F_2 population. Means separations for different genotypes of SAP6 are presented in Table 3. For both populations, the predominant trend was that no significant differences in CBB reaction were observed among the three genotypes. However, for the Othello/ABCP-8 population, SAP6 SAP6 had a significantly lower CBB reaction at 14 DAI than SAP6 sap6. For the Red Hawk/USDK-CBB-15 population, SAP6 sap6 genotypes had a significantly lower CBB reaction at 18 DAI than sap6 sap6.

Discussion

Two F_2 populations that segregated for resistance to CBB were examined in this study. One was a pinto bean population derived from a cross between Othello (susceptible) × ABCP-8 (resistant) and the second was a dark red kidney bean population

resulting from a cross between Red Hawk (susceptible) × USDK CBB-15 (resistant). The genotypes of each plant for loci corresponding to two dominant SCAR markers, SAP6 and SU91, were co-dominantly interpreted simultaneously using a multiplex real-time PCR reaction.

Disease reactions to CBB were evaluated in F_2 plants and reactions were compared among all nine possible genotypic classes (Tables 1, 2). Reactions to CBB were also compared separately for the three possible genotypes for SAP6 and SU91 (Table 3). Similar results were observed for both F_2 populations in that at every time point the three homozygous recessive SU91 genotypes (sap6 sap6//su91 su91); (SAP6 sap6//su91 su91), and (SAP6 SAP6//su91 su91) had significantly higher mean reactions (more susceptible) to CBB than the six other genotypes (Tables 1, 2). Across all time points for both F_2 populations, significant differences in CBB reaction were rarely observed among the six genotypes with at least a single copy of SU91 (_ _//SU91 _). CBB reactions were also compared separately for all three possible genotypes for SAP6 and SU91 (Table 3). The mean CBB reaction of plants that were homozygous recessive for SU91 (su91 su91) was significantly higher (more susceptible) than either SU91 su91 or SU91 SU91 genotypes for both F_2 populations at each time point (Table 3). In addition, for both F_2 populations, at each time point no significant differences in CBB reaction were observed between SU91 SU91 and SU91 su91 genotypes. Differences in CBB reaction among the three different genotypes for SAP6 were predominately not significant (Table 3). The pairwise combinations of SU91 genotype classes for which significant differences in CBB reaction were observed were not consistent across both populations.

The results suggest that reaction to CBB caused by *Xap* isolates Xf260 and Xf410 in these two F_2 populations is primarily conditioned by dominant effects of SU91 and that SAP6 does not appreciably influence reaction to CBB in these two populations. Nonetheless, an examination of phenotypic segregation for reaction to CBB suggests that this interaction does not fit expected segregation ratios for a single dominant gene. In the case of the pinto F_2 population (Othello/ABCP-8), segregation of resistance was significantly different than the expected 3 resistant:1 susceptible ratio ($\chi^2 = 8.02$; $df = 1$; $P = 0.005$). A comparison of CBB reactions at 14 DAI between Othello, ABCP-8, and their F_2 progeny indicates that ABCP-8 had a resistant reaction (CBB $\leq$ 3.0), Othello a susceptible reaction (CBB $\geq$ 7), and the six _ _//SU91 _ genotypes had intermediate (3 <CBB <7) CBB reactions (Table 1). Different disease reactions between ABCP-8 and F_2 plants with the SAP6 SAP6/SU91 SU91 genotype suggest that the intermediate reaction observed at 14 DAI in the six _ _//SU91 _genotypes may be due to segregation of other QTL that contribute to the high level of resistance in ABCP-8.

Segregation for resistance at 18 DAI in the Red Hawk × USDK-CBB-15 F_2 population also deviated significantly from the expected 3 resistant:1 susceptible ratio ($\chi^2 = 8.60$; $df = 1$; $P = 0.003$). At 14 DAI and 18 DAI USDK-CBB-15 exhibited an intermediate CBB reaction (3 <CBB <7), as did F_2 plants with _ _//SU91 _ genotypes derived from the cross Red Hawk × USDK-CBB-15, while the susceptible parent Red Hawk exhibited a susceptible CBB reaction (CBB $\geq$ 7; Table 2). The similar reactions of USDK-CBB-15 and the _ _//SU91 _ F_2 plants suggests that resistance to CBB in this cross was not appreciably influenced by other QTL beside SU91.

Although SAP6 did not contribute to resistance in either of the two crosses examined in this study, the marker has been associated with resistance to CBB in other populations (Miklas et al. 2003). The lack of effect of SAP6 towards resistance to CBB could be due to recombination between the SAP6 marker and the QTL conditioning resistance. SAP6 has been previously detected in bean cultivars such as Matterhorn, which is susceptible to CBB (Miklas et al. 2003). The results presented in this report suggest that resistance to CBB cannot be assumed to be present simply based on the detection of SAP6 in a plant genome.

Differences between this report and previous investigations on the role of SAP6 in resistance to CBB may reflect specificity of SAP6 towards distinct races of *Xap* or differences in environmental conditions that influence the severity of CBB reaction. Although a set of differential common bean lines has not been identified to distinguish specific races of *Xap*, race specificity of isolates of *Xap* towards tepary bean lines has been repeatedly observed (Zaiter et al. 1989; Opio et al. 1996). Mutlu et al. (2008) recently

examined a range of isolates of *Xap* for the ability to cause disease on 13 different common bean genotypes. Significant sources of variation on reaction to CBB included pathogen isolate, bean genotype, and isolate × bean genotype interaction. Isolates from Africa were more pathogenic on the bean genotypes tested than were isolates from North America (Mutlu et al. 2008). Mkandawire et al. (2004) also observed differences in pathogenicity on a wide range of common bean genotypes between African isolates of *X. axonopodis* and an isolate from Puerto Rico.

The resistant parent ABCP-8 was developed based on screening for CBB reaction using the *Xap* isolates DR-7, from the Dominican Republic, and EK-11, from Nebraska (Mutlu et al. 2005). The ABCP-8 plants inoculated with these two isolates exhibited 6% infection, whereas in our study ABCP-8 plants exhibited disease scores below 3 within 10 DAI (data not shown) which equates to about 15% infection. The resistant parent USDK-CBB-15 was initially evaluated using *Xap* isolates from N. America, and the mean CBB reaction based on greenhouse inoculations ranged from 2.0 to 3.6 (Miklas et al. 2006a, 2006b). In our study, within 14 DAI the mean CBB reaction of USDK-CBB-15 was >3.0 (Table 2), which is characteristic of an indeterminate disease reaction as opposed to a resistant response. These results suggest that the disease screening employed in our study, which involved the artificial inoculation of plants in the greenhouse with African *Xap* isolates Xf260 and Xf410, resulted in more severe disease than was observed during the screening used initially to develop ABCP-8 (Mutlu et al. 2005) and USDK-CBB-15 (Miklas et al. 2006a, 2006b). It is possible that this severe disease environment masked any effect SAP6 might have on resistance to CBB in the two F_2 populations examined in this study.

Different combinations of *Xap* isolates and bean populations have also resulted in different results concerning how QTL interact to condition resistance to CBB. O'Boyle et al. (2007) examined the interaction between SU91 and BC420 in the expression of resistance to CBB using isolate 9712-3, from Nebraska, and 93 $F_{3:4}$ plants derived from a cross between the black bean breeding line 99L91-45 and HR45/Kaboon. Genotypes that possessed both BC420 and SU91 had less resistance to CBB than genotypes that possessed only one of either marker and suggested the possibility of an epistatic interaction between the two QTLs. Vandemark et al. (2008) examined the interaction between BC420 and SU91 using the African *Xap* isolates Xf260 and Xf410 on 120 BC_6:F_2 plants derived from a cross between XAN 159 (resistant) and Teebus (susceptible). Reaction to CBB in BC_6:F_2 plants was characterized by an epistatic interaction between BC420 and SU91 in which plants that had at least a single copy of BC420 but were homozygous recessive for SU91 were susceptible to CBB; plants that had at least one copy of SU91 but were homozygous recessive for BC420 expressed an intermediate disease reaction, and the highest level of disease resistance was conferred by genotypes with at least a single copy of both SU91 and BC420 (Vandemark et al. 2008).

Unfortunately, SAP6 is absent in both XAN 159 (Miklas et al. 2003) and Teebus, so the XAN 159 × Teebus population could not be used to examine the interaction of SAP6 and SU91. Consequently, two other F_2 populations that segregated for SAP6 and SU91 were challenged with *Xap* isolates Xf 260 and Xf 410, which were also used to examine the interaction of SU91 and BC420 in a XAN 159 × Teebus population. Our results demonstrate that in the two F_2 populations examined in this study resistance to CBB caused by the *Xap* isolates Xf260 and Xf410 was primarily conditioned by dominant effects of SU91 and that SAP6 did not appreciably influence reaction to CBB in these two populations. However, comparisons between our results and previously published results suggests that the role of specific QTL and interactions between QTL on the expression of resistance to CBB can be influenced by the population examined and the isolates of *X. axonopodis* used to infect plants. The development of a set of *P. vulgaris* differentials that could clearly distinguish pathogenic races of *Xap* would facilitate the examination of the role of specific QTL in disease resistance across a range of common bean populations.

References

Aggour AR, Coyne DP, Vidaver AK (1989) Comparison of leaf and pod disease reactions of beans (*Phaseolus vulgaris* L.) inoculated by different methods with strains of *Xanthomonas campestris* pv. *phaseoli* (Smith) Dye. Euphytica 43:143–152

Andrus CF (1948) A method of testing beans for resistance to bacterial blights. Phytopathology 38:757–759

Beebe SE (1989) Quantitative genetics in *Phaseolus vulgaris*: the example of resistance to *Xanthomonas campestris* pv. *phaseoli*. In: Beebe S (ed) Current Topics in Breeding of Common bean. Proc. Int. Bean Breeding Workshop Working Doc. No 47:213-238. CIAT, Cali, Columbia

Burke DW, Silbernagel MJ, Kraft JM, Koehler HH (1995) Registration of 'Othello' pinto bean. Crop Sci 35:943

Coyne DP, Schuster ML (1974) Inheritance and linkage relations of reaction to *Xanthomonas phaseoli* (E. F. Smith) Dowson (common blight), stage of plant development and plant habit in *Phaseolus vulgaris* L. Euphytica 23:195–204

Heid CA, Stevens J, Livak KJ, Williams PM (1996) Real time quantitative PCR. Genome Res 6:986–994

Holland PM, Abramson RD, Watson R, Gelfand D (1991) Detection of specific polymerase chain reaction products by utilizing the 5′-3′ exonuclease activity of *Thermus aquaticus* DNA polymerase. Proc Natl Acad Sci USA 88:7276–7280

Kelly JD, Hosfield GL, Varner GV, Uebersax MA, Afanador LK, Taylor J (1998) Registration of 'Red Hawk' dark red kidney bean. Crop Sci 38:280–281

Kolkman JM, Michaels TE (1994) Major gene control of common bacterial blight in *Phaseolus vulgaris*. Annu Rep Bean Improv Coop 37:73–74

Miklas PN, Johnson E, Stone V, Beaver JS, Montoya C, Zapata M (1996) Selective mapping of QTL conditioning disease resistance in common bean. Crop Sci 36:1344–1351

Miklas PN, Smith JR, Riley R, Grafton KF, Singh SP, Jung G, Coyne DP (2000) Marker-assisted breeding for pyramided resistance to common bacterial blight in common bean. Annu Rep Bean Improv Coop 43:39–40

Miklas PN, Coyne DP, Grafton KF, Mutlu N, Reiser J, Lindgren T, Singh SP (2003) A major QTL for common bacterial blight resistance derives from the common bean great northern landrace cultivar Montana No. 5. Euphytica 131:137–146

Miklas PN, Kelly JD, Beebe SE, Blair MW (2006a) Common bean breeding for resistance against biotic and abiotic stresses: from classical to MAS breeding. Euphytica 147:105–131

Miklas PN, Smith JR, Singh SP (2006b) Registration of common bacterial blight resistant dark red kidney bean germplasm line USDK-CBB-15. Crop Sci 46:1005–1007

Mkandawire ABC, Mabagala RB, Guzman P, Gepts P, Gilbertson RL (2004) Genetic diversity and pathogenic variation of common blight bacteria (*Xanthomonas campestris* pv. *phaseoli* and *X. campestris* pv. *phaseoli* var. *fuscans*) suggests pathogen co-evolution with the common bean. Phytopathology 94:593–603

Mutlu N, Miklas PN, Steadman JR, Vidaver AK, Lindgren DT, Reiser J, Coyne DP, Pastor-Corrales MA (2005) Registration of common bacterial blight resistant germplasm line ABCP-8. Crop Sci 45:806–807

Mutlu N, Vidaver AK, Coyne DP, Steadman JR, Lambrecht PA, Reiser J (2008) Differential pathogenicity of *Xanthomonas campestris* pv. *phaseoli* and *X. fuscans* subsp. *fuscans* strains on bean genotypes with common blight resistance. Plant Dis 92:546–554

O'Boyle PD, Kelly JD, Kirk WW (2007) Use of marker assisted selection to breed for resistance to common bacterial blight in common bean. J Amer Soc Hort Sci 132:381–386

Opio AF, Allen DJ, Teri JM (1996) Pathogenic variation in *Xanthomonas campestris* pv. *phaseoli*, the causal agent of common bacterial blight in *Phaseolus* beans. Plant Pathol 45:1126–1133

Paran I, Michelmore RW (1993) Development of reliable PCR-based markers linked to downy mildew resistance genes in lettuce. Theor Appl Genet 85:985–993

Park SO, Coyne DP, Mutlu N, Jung G, Steadman JR (1999) Confirmation of molecular markers and flower color associated with QTL for resistance to common bacterial blight in common beans. J Amer Soc Hort Sci 124:519–526

Pedraza F, Gallego G, Beebe S, Tohme J (1997) Marcadores SCAR y RAPD para la resitencia a la bacteriosis comun (CBB). In: Singh SP, Voysest O (eds) Taller de mejoramiento de frijol para el Siglo XXI: bases para una estrategia para America Latina. CIAT, Cali, Columbia, pp 130–134

Saettler AW (1991) Common Bacterial Blight. In: Hall R (ed) Compendium of bean diseases. American Phytopathological Society Press, St. Paul, MN, pp 29–30

Shapiro SS, Wilk MB (1965) An analysis of variance test for normality (complete samples). Biometrika 52:591–611

Silva LO, Singh SP, Pastor-Corrales MA (1989) Inheritance of resistance to bacterial blight in common bean. Theor Appl Genet 78:619–624

Singh SP, Munoz CG (1999) Resistance to common bacterial blight among Phaseolus species and common bean improvement. Crop Sci 39:80–89

Thomas CV, Waines JG (1984) Fertile backcross and allotetraploid plants from crosses between tepary beans and common beans. J Hered 75:93–98

Vandemark GJ, Miklas PN (2002) A fluorescent PCR assay for the codominant interpretation of a dominant SCAR marker linked to the virus resistance gene $bc\text{-}1^2$ in common bean. Molecular Breed 10:193–201

Vandemark GJ, Miklas PN (2005) Genotyping common bean for the potyvirus resistance alleles *I* and $bc\text{-}1^2$ with a multiplex real-time PCR assay. Phytopathology 95:499–505

Vandemark GJ, Fourie D, Miklas PN (2008) Genotyping with real-time PCR reveals recessive epistasis between independent QTL conferring resistance to common bacterial blight in dry bean. Theor Appl Genet 117:513–522

Walpole RE, Myers RH (1978) Probability and statistics for engineers and scientists, 2nd edn. Macmillan, New York, pp 113–122

Weller DM, Saettler AW (1976) Chemical control of common and fuscous bacterial blights [*Xanthomonas phaseoli*, *Xanthomonas phaseoli* fuscans] in Michigan navy (pea) beans. Plant Dis Rept 60:793–797

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingley SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nuc Acids Res 18:6531–6535

Yu ZH, Stall RE, Vallejos CE (1998) Detection of genes for resistance to common bacterial blight of beans. Crop Sci 38:1290–1296

Zaiter HZ, Coyne DP, Vidaver AK, Steadman JR (1989) Dirrenetial reactions of tepary bean to *Xanthomonas campestris* pv. *phaseoli*. HortScience 24:134–137